DIURNAL CHANGES IN THE ELECTRICAL RESPONSE OF THE COMPOUND EYE[1]

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Diurnal changes in the physical environment have resulted in the establishment of biological diurnal rhythms in a large number of organisms. Many of these biological rhythms are apparently under the direct control of the environment for they disappear as soon as the organism is subjected to a constant environment. Others, however, appear to be inherent rhythms for they continue for long periods of time after the organism is placed in a constant environment. Welsh (1938), in his review of the rather extensive literature on this subject, has cited numerous examples of persistent diurnal rhythms and has discussed the possible mechanisms that might be involved in the control of such rhythms.

While engaged in a study of the electrical potentials associated with illumination of the compound eye of certain arthropods, the authors made the interesting observation that the magnitude and form of the electrical response from the eye of the carabid beetle, *Chlaenius diffinis,* vary according to the time of day, even though the animal was kept continuously in the dark. Further investigation disclosed that such daily changes in the potentials from the eyes occurred in a number of other beetles. It is the purpose of this report to describe these diurnal fluctuations in potential and to correlate them with other types of diurnal changes that are known to occur in the eyes of certain invertebrates.

Materials and Methods

For this investigation the following beetles were employed: *Chlaenius diffinis, Chlaenius tomentosus, Hydrus triangularis, Harpalus pennsylvanicus, Harpalus caliginosus, Anomoglossus emarginatis, Osmoderma eremicola,* and *Necrophorus orbicollis.*[2] A number of experiments were also performed with the crayfish, *Cambarus virilis.* All these animals were obtained directly from the field and, with few exceptions, were

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used within a few days after being brought into the laboratory. The experiments were all performed between May and October, 1939.

The method of obtaining and recording the potentials from the eye is similar to that described in connection with studies on the grasshopper (Jahn and Crescitelli, 1938). The method consists in leading off from the corneal surface of one eye by means of a silver-silver chloride electrode. The electrode dips into a reservoir of physiological solution which, in turn, is connected by means of a small glass siphon to a solution-filled chamber built around the eye. The front of this eye-chamber is transparent and permits illumination of the eye. The other eye is prepared in the same manner except that the front of the chamber is opaque. This permits a study of the potential which develops across the two eyes when one eye is illuminated. The intensity of the light striking the eye and the exposure time is controlled and known in all cases. The potential, after amplification through a condenser-coupled amplifier (time constant of 1.9 seconds), is recorded by means of a cathode ray oscillograph and camera.

To demonstrate the occurrence of a diurnal rhythm in the electrical response, the animal, prepared in the above manner, is placed in a darkened moist chamber and kept there throughout the course of the experiment. At intervals of an hour or two throughout the day and evening a flash of bright light (725 foot candles) is admitted to the eye and the resulting deflection of the cathode ray beam is photographed. In some animals it is possible to continue the experiment for 3–4 days; in others, however, because of the limited life of the animal under the conditions of the experiment, only a 24-hour run is possible. Temperatures were recorded with a resistance thermometer throughout a number of the experiments, and it was found that either the temperature remained approximately constant or that the variations did not coincide with variations in the electrograms. Reference to barograms which were recorded during the period of the present experiments showed that small diurnal changes in barometric pressure did tend to occur but that this tendency was very often masked by much larger progressive variations which had no relation to the diurnal cycle.

General Description of the Electrical Response of the Arthropod Eye

The form and magnitude of the electrical response which is obtained by illumination of the compound eye of arthropods varies with the intensity of illumination, the length of the exposure period, the degree of light- or dark-adaptation of the eye, and the particular species of animal that is involved (Jahn and Crescitelli, 1938; 1939). For the grasshopper (*Melanoplus differentialis*) the electrical response of the dark-

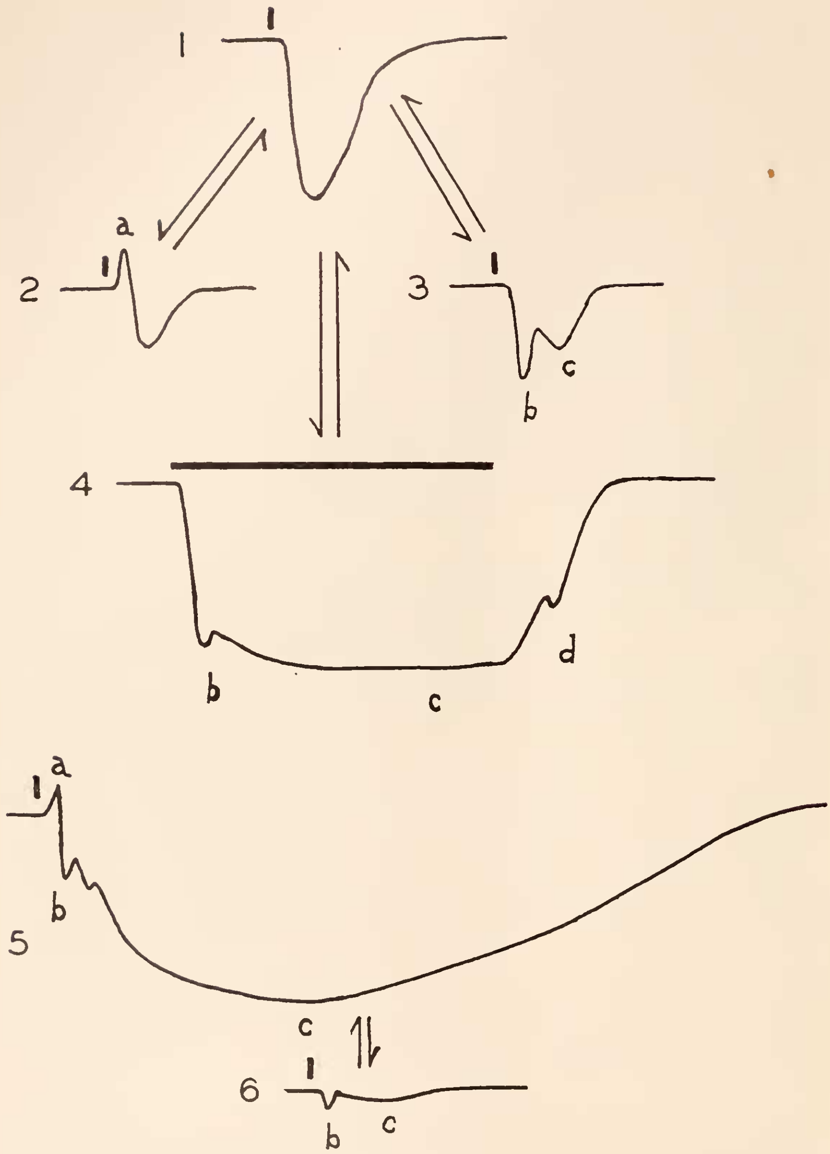

FIG. 1. Diagrams of typical electrical responses. Record 1, dark-adapted grasshopper, brief flash of high intensity; record 2, dark-adapted grasshopper, brief flash, low intensity; record 3, light-adapted grasshopper, high intensity, brief exposure; record 4, light-adapted grasshopper, high intensity, one second exposure; record 5, dark-adapted Cecropia moth, high intensity, brief exposure; record 6, light-adapted Cecropia moth, high intensity, brief exposure.

adapted eye to a brief exposure of bright light is of a relatively simple type (Record 1, Fig. 1). It consists of a rapid deflection (downward) indicating the development of negativity at the illuminated eye with respect to the non-illuminated eye. Following this is a relatively slow decline of the potential. This wave form is somewhat more complex when the intensity of the stimulating light is low (Record 2, Fig. 1) or when the eye is light-adapted (Record 3, Fig. 1). In the Cecropia moth (*Samia cecropia*) the typical response of the dark-adapted eye to a brief exposure of bright light is that shown in Record 5, Fig. 1. This response is obviously polyphasic, consisting of a number of elements, the most typical being the a-, b-, and c-waves. The a-wave is a small, spike-like deflection which indicates a condition of positivity of the illuminated eye with relation to the other eye. Following the a-wave is the rapid negative deflection known as the b-wave while the c-wave is the very slow and prominent terminal phase. In addition to these three elements there may be several poorly characterized deflections located between the b- and c-waves. Light-adaptation of the eye results in abolishment of the a-wave, a reduction in magnitude of the b-wave, and a reduction in both magnitude and duration of the c-wave (Record 6, Fig. 1). Electrograms similar to those obtained from the Cecropia moth have also been noted in the Luna moth (*Actias luna*), the Promethea moth (*Callosamia promethea*), and other moths. The electrical responses of the grasshopper and Cecropia moth eyes have been studied between the hours of 7:00 A.M. and midnight on numerous occasions, and in no case was any evidence obtained that the magnitude and wave form of the response varied with the hour of the day.

Results and Discussion

Evidence of a Diurnal Rhythm in Beetles

Diurnal fluctuations in the electrical response have been obtained with the following beetles: *Chlaenius diffinis* (6), *Chlaenius tomentosus* (1), *Harpalus pennsylvanicus* (2), *Harpalus caliginosus* (1), and *Hydrus triangularis* (3). The numbers in parentheses indicate the number of animals of each species that were used. Records 1–30, inclusive, Fig. 2, demonstrate the diurnal changes as they occur typically in *Chlaenius diffinis*. This animal was studied for four consecutive days. (The records obtained on the first day, which are identical with those of the second day, are not included in this group because they are used later (Records 67–77, Fig. 3) in the discussion of another group of results.) Record 1, which is the response to a brief exposure, was photographed at 9:20 A.M. of the second day. The response is fairly simple in nature and not greatly unlike that obtained from the grasshopper eye. The same type of response was elicited from the eye during the morning and early afternoon hours (Records 2–5). At 3:57 P.M. (Record 6) a distinctly different type of electrogram was obtained. The wave form of this record is polyphasic, consisting of an initial series of relatively

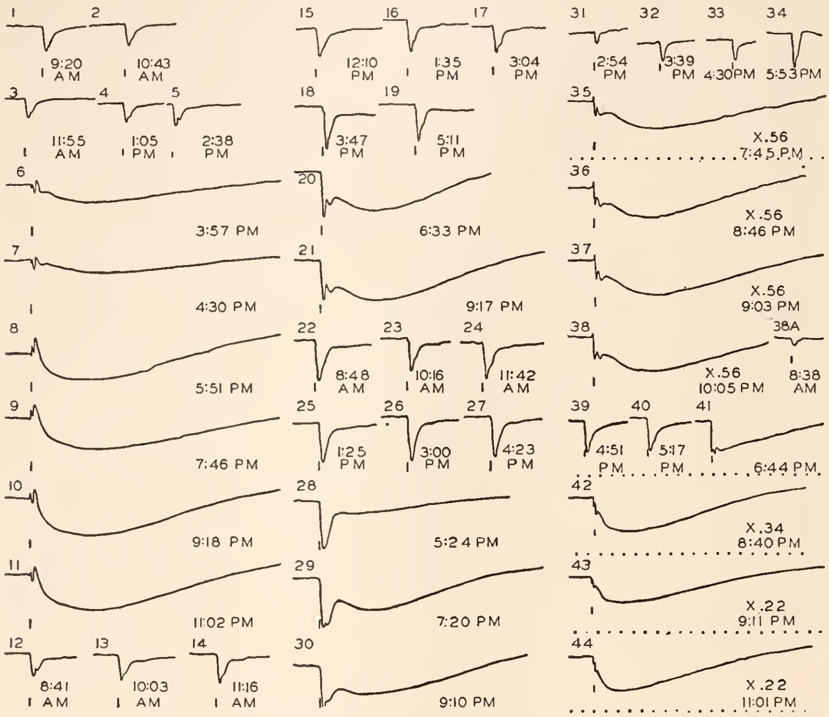

FIG. 2. Electrical responses from the eyes of certain beetles at different hours during the day and evening. All responses are to brief exposures of a bright (725 f.c.) light. The records shown are typical responses selected from many which comprised each series. All records of Figs. 2 and 3 were taken with the recording paper moving at the same speed. Distances between dots of the time record, when shown, indicate 142 sigma. The records for each animal were taken with the same amplifier gain except when indicated as X. These and the following records have been retouched to facilitate reproduction. Refer to text for further details.

rapid waves and a long slow phase (c-wave). From 3:57 P.M. to 11:02 P.M. (Records 6–11) this complex wave form persisted, and the slow wave increased in magnitude. On the morning of the third day of the experiment (Record 12) a brief exposure of the eye to light again elicited the relatively simple type of electrogram. This persisted with slight modifications until 5:11 P.M. (Records 13–19). At 6:33 P.M.

of the third day (Record 20) the relatively complex wave form was again obtained although in this case it was somewhat different from the polyphasic wave of the first and second evenings, indicating a progressive change with time. The simple wave form was once more recorded on the morning of the fourth day (Record 22) and persisted throughout that day (Records 23–27). At 5:24 P.M. (Record 28) a brief exposure to light called forth a response showing the beginning of a slow wave, and at 7:20 P.M. (Record 29) and 9:10 P.M. (Record 30) polyphasic electrograms were again recorded.

The diurnal rhythm in another specimen of *Chlaenius diffinis* is presented in Records 31–38A (Fig. 2), inclusive. During the afternoon (Records 31–34) the responses obtained from this animal were of the typically simple type. During the evening hours (Records 35–38) a complex type of wave form, not greatly unlike that obtained from certain moths, was recorded. These electrograms possess the a-, b-, and c-waves described in connection with the Cecropia moth (Fig. 1). The next day (Record 38A) the typical day-type of response was once again obtained. A series of records from still another specimen of *Chlaenius diffinis* are shown in Fig. 3 (Records 85–96, inclusive). In this case the complete change from the night-type to the day-type did not occur until the late morning hours of the second day. Since the animal was found dead late in the afternoon, it seems as if the delay in the change of the response to the day-type might have been associated with the degenerative changes occurring in the animal.

The electrical responses from the eye of the water beetle, *Hydrus triangularis,* also undergo marked diurnal variations in both form and magnitude (Records 60–66, 97–107; Fig. 3). In many respects the diurnal changes that occur in this beetle are not greatly unlike those that occur in *Chlaenius diffinis.* The day-type of response is here also relatively simple while the night-type of response is polyphasic and contains the characteristic slow c-wave.

A diurnal change in the potential from the eye occurs also in the carabid beetle, *Chlaenius tomentosus* (Records 45–59, inclusive, Fig. 3). Here also the day-type of response (48–51, 59) is relatively simple and lacking in any distinct slow component. The night-type of response is again larger in magnitude, polyphasic, and characterized by the presence of a definite slow wave. Incidentally, it may be noted that the night-type of response which was obtained during the first evening (45–47) is different from the response which was photographed during the second evening (52–58), indicating a change with time.

In two other species of carabid beetles, *Harpalus caliginosus* and *Harpalus pennsylvanicus,* it has been found that a rapid and relatively

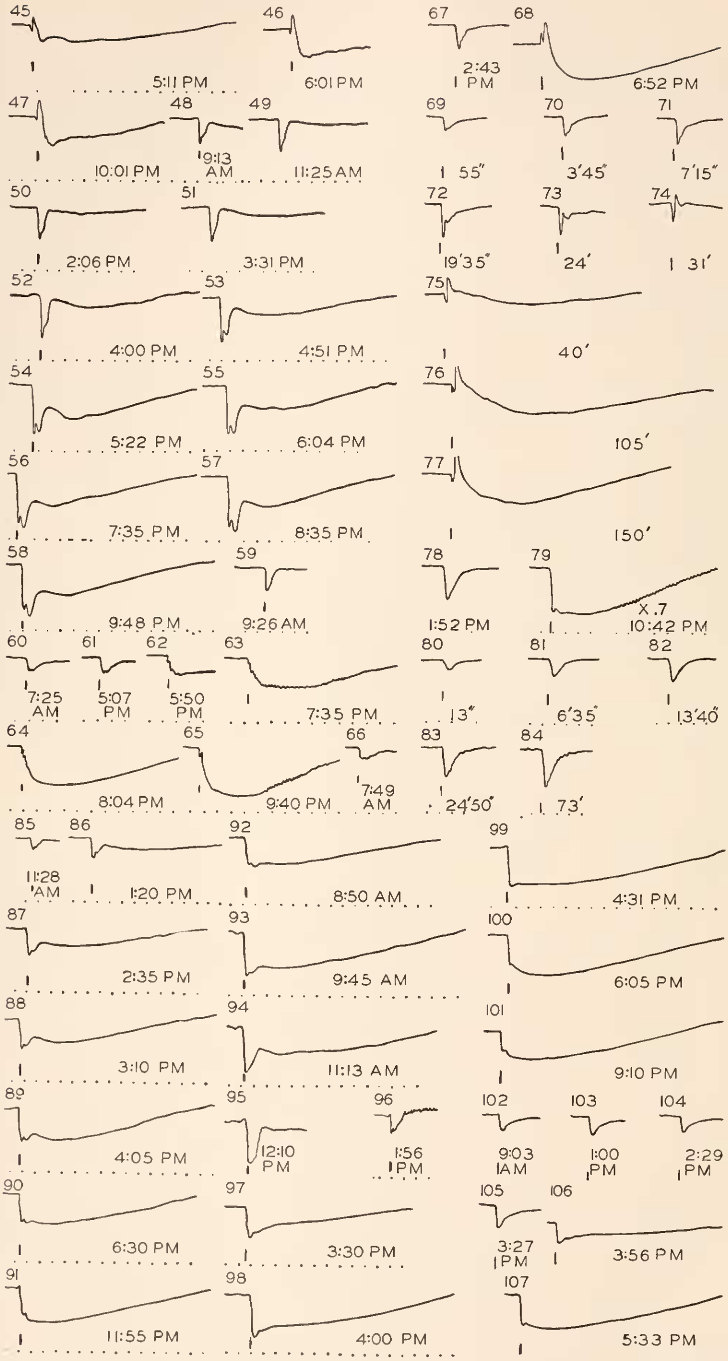

FIG. 3. Electrical responses from the eyes of certain beetles at different hours of the day and evening (Records 45–66, 85–107) and the effect of light adaptation on the response (Records 67–84). For further details refer to text.

simple type of response is obtained during the morning and afternoon hours, whereas a much larger and relatively complex type of electrogram is characteristic of the evening hours. Records 39–44 (Fig. 2) illustrate these changes for *Harpalus caliginosus.*

It is clear from these results that a diurnal variation exists in the electrical response from the eyes of a number of species of beetles when the animals are kept in total darkness. There is a characteristic day-type of response present in all the beetles studied which is significantly different in form and magnitude from the typical night-type of response. The transformation from the day-type to the night-type of response occurs generally between 5:00 P.M. and 7:00 P.M. although in a number of cases the beginning of the change occurred as early as 3:30 P.M. The exact time at which the change occurs from the night-type to the day-type of electrogram has not been determined. In one beetle (*Chlaenius diffinis*) the change took place between 4:00 A.M. and 7:00 A.M. Not enough all-night studies have been made of this point, however, to warrant any conclusions.

A few experiments have been made on beetles which, apparently, do not possess a diurnal rhythm of the type described here. In one experiment on *Anomoglossus emarginatus,* one experiment on *Osmoderma eremicola,* and one experiment on *Necrophorus orbicollis,* no indications of diurnal fluctuations in the electrical potentials were obtained. This paper is not intended to be a survey of the beetles, however, and many more species will have to be examined before any generalizations and conclusions may be made regarding the significance of the presence or absence of a diurnal rhythm.

Effect of Light-Adaptation

If the dark-adapted eye of a beetle which is in the night-phase of its rhythm is light-adapted, the magnitude of the response to a single brief exposure is reduced markedly and the form of the response is altered. If this eye is then allowed to dark-adapt, the original magnitude and wave form slowly return. These facts are illustrated for *Chlaenius diffinis* in Records 67–77 (Fig. 3). In this series Records 67 and 68 show respectively the day-type and night-type of responses of the dark-adapted eye taken respectively at 2:43 P.M. and at 6:52 P.M. of the same day. At 7:00 P.M. the eye, when in the night-phase of its cycle, was light-adapted by exposure to a 725 foot-candle light for 15 minutes. Record 69, which was made 55 seconds after cessation of light-adaptation, shows the response of this light-adapted eye to a single brief exposure. The wave form has changed and the magnitude of the potential has been reduced. Records 70–77 show the gradual restoration of the night-type of response. The restoration is rather slow for even after 150 minutes of dark-adaptation (Record 77) the original magnitude and form of Record 68 had not been restored. (The electrograms which were obtained the following night (Records 8–11, Fig. 2) were almost identical in wave form with that of Record 68, Fig. 3.)

Light-adaptation of a dark-adapted eye which is in the day-phase of its cycle results in a reduction in magnitude but not in an appreciable alteration of wave form. Records 78–84 demonstrate this for *Hydrus triangularis*. In this series the day-type and night-type of responses were recorded respectively at 1:52 P.M. (Record 78) and at 10:42 P.M. (Record 79) of the same day. At 2:40 P.M. the eye, when still in the day-phase of its cycle, was light-adapted and the response (Record 80) taken 13 seconds after cessation of light-adaptation, illustrates the effect of the light-adaptation. Recovery from light-adaptation is shown in Records 81–84. Seventy-three minutes after cessation of light-adaptation (Record 84) the original magnitude of the dark-adapted eye had been restored.

Interpretation and Conclusions

Although a considerable number of investigations have appeared on the electrical phenomena of the vertebrate and invertebrate eyes (for literature refer to Granit, 1936; Jahn and Crescitelli, 1938, 1939), this appears to be the first report of a diurnal rhythm in the electrical response of the eye. Since the origin of the potentials in the eye is not known, it is impossible to deduce from the potentials alone the nature of the diurnal changes occurring in the eye that are responsible for the diurnal changes in the electrical responses. There are a number of already well established facts, however, that may be correlated with the diurnal variations in electrical potentials. It has been known for some time that persisting diurnal changes in the position of the distal and proximal pigments occur in the eyes of certain arthropods (Welsh, 1938). Such rhythmic changes in the position of the pigment have not been observed in any of the beetles studied, but it is possible that such changes do occur and may be related to the observed diurnal variations in the electrical response. It is known that the form and magnitude of the response is modified by light- and dark-adaptation of the eye (Jahn and Crescitelli, 1938, 1939) as is also the position of the proximal and distal pigments (Parker, 1932). One way of resolving this question would be to study the electrical responses in an animal in whose eyes are definitely known to occur daily rhythmic migrations of the pigment. The crayfish (*Cambarus virilis*) is one in which the position of the proximal pigment is known to vary with the time of day (Bennitt, 1932). We have experimented with a number of freshly caught crayfish whose eyes showed a persisting diurnal rhythm in glow, but in no case were we able to obtain definite evidence of a diurnal rhythm in the electrical response. Studies which are in progress on the morphology of some of the beetle eyes used in this investigation may be of some assistance in reaching a tentative conclusion regarding the relationship between the character of the response and the position of the pigment.

The mechanism which is in primary control of these diurnal periodic changes is not known. The suggestion has often been made of the existence of an inherent physiological process operating independently of the environment and acting as a timing mechanism (Welsh, 1938). In the present experiments a number of environmental factors can be dismissed as controlling factors. These are illumination, moisture, temperature and barometric pressure, all of which were either controlled or eliminated as explained under "Materials and Methods." Ionization of the air has apparently been eliminated by Horstmann (1935) as a determining influence in the diurnal migration of the eye pigment in certain moths. The possibility that cosmic radiation may be the controlling factor is rendered unlikely since Millikan (1932) found little, if any, difference in the intensity of this radiation at different times during the day. There is always the possibility that some other environmental influence, as yet unsuspected, may serve as the stimulus for the electrical rhythm that has been observed. Until such an influence is found, however, we must conclude that an internal mechanism acting independently of the immediate environment lies at the basis of the electrical rhythm.

Summary

1. A diurnal rhythm occurs in the electrical responses obtained from the compound eyes of certain beetles (*Chlaenius diffinis, Chlaenius tomentosus, Hydrus triangularis, Harpalus caliginosus, Harpalus pennsylvanicus*). When one of these beetles is kept in total darkness and under approximately constant environmental conditions, the electrical response to a brief exposure of light which is recorded during the morning and afternoon hours (day-type) is markedly different from the response obtained during the late afternoon and evening hours (night-type).

2. The day-type of record is relatively simple and not unlike that which is always obtained from certain grasshoppers and butterflies. The night-type of response, always of greater magnitude, possesses a complex wave form similar in many respects to that always elicited from the eyes of certain moths.

3. The day-type of response is reduced in magnitude but not greatly altered in form when the eye is light-adapted. The night-type of electrogram is not only reduced in magnitude by light-adaptation of the eye, but the wave form is markedly altered.

4. The possibility is suggested that the diurnal cycle in the electrical response may be related to a diurnal migration of eye pigments.

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